Subject: Re: Biological Agents Registry Form
From: Stephen Barr <Stephen.Barr@schulich.uwo.ca>
Date: Thu, 26 Jan 2012 11:48:17 -0500
To: jtorchia@uwo.ca

New Info (letter only)

Dear Dr. Torchia,

My name is Steve, I am one of the virologists sitting on the Biohazardous Subcommittee. We as a Subcommittee are having a difficult time understanding exactly what your lab intends to do regarding the two genes (CIP and ZNF217), which your BARF indicates are potential oncogenes. Our concern stems from a biohazardous safety point of view for your workers who will be handling these genes, in accordance to health safety regulations set by the Public Health Agency of Canada (PHAC). When asked to explain the biohazards associated with your proposed research, there was no explanation about the intended use of your Adenoviruses. Our concern is that they will be used to transduce CIP or ZNF217, which are potentially oncogenic. Do you intend to use Adenovirus to transduce CIP or ZNF217 or any other gene? If so, what precautions are you taking to ensure the safety of workers carrying out this procedure? If you will be using Adenovirus to transduce potential oncogenes, the Subcommittee will need to perform a risk assessment in consultation with PHAC regarding the proper Biosafety Containment Level for this work. With the use of potential oncogenes, the containment level could be classified as level 2+ or level 3. If you are not using Adenovirus to transduce ANY genes, we would like a statement stating that, and what the virus will be used for.

Furthermore, in your current BARF, we noticed several inconsistencies that have raised a flag. In your summary of the research proposed, you have stated that CIP and ZNF217 are potential oncogenes, however in section 4.2 of your BARF, you state "the constructs generated and used in our laboratory are not oncogenic." In section 4.6, it should be checked "yes" for "Will virus be infectious to humans or animals" since Adenoviruses are infectious. In Table 1.2, Adenovirus is listed , however it was not checked as to whether it is a human pathogen, animal pathogen or zoonotic. Table 4.3, "Will genetic modifications involving viral vectors be made?", was checked no. This is okay as long as you are not using Adenoviruses to transduce genes. In our correspondence with Majdina, we were confused by the statement that "Adenovirus does not have the capability to infect regular human cells," which is incorrect. In Section 4.4, the use of SV40 large T antigen was checked "no", however this should be checked "yes" since your lab uses 293T cells, which contain SV40 large T antigen.

It is not the intention of the Subcommittee to unnecessarily delay approval for your research. Our frustration stems from the PERCEIVED lack of concern for details in the BARF that could put workers at significant risk for their health safety. Part of our job is to ensure that all members of the lab are aware of the risks, are taking the appropriate precautions to mitigate these risks, and are acting in accordance to policies established by PHAC. I hope that this clears up any misunderstanding and that it is clear what information the Subcommittee needs regarding your BARF. Please do not hesitate to contact me directly if you need further clarification. Thanks in advance.

Steve
(on behalf of the Biohazards Subcommittee)

Stephen D. Barr, PhD
The University of Western Ontario.
Department of Microbiology and Immunology.
Schulich School of Medicine and Dentistry, Centre for Human Immunology.
Dental Sciences Building Room 3006B
London, Ontario

N6A 5C1
CANADA
Phone: 519-661-3438
Main webpage:
http://publish.uwo.ca/~sbarr9/barrlab.html

Text only webpage: http://publish.uwo.ca/~sbarr9/barrlab2.html

THE UNIVERSITY OF WESTERN ONTARIO
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: July 9, 2010
Biosafety Website: www.uwo.ca/humanresources/biosafety/

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario (UWO) or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biological agents is described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans or involving plants, fungi, or insects that require Public Health Agency of Canada (PHAC) or Canadian Food Inspection Agency (CFIA) permits.

This form must be updated at least every 3 years or when there are changes to the biological agents being used.

Containment Levels will be established in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Public Health Agency of Canada (PHAC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

Completed forms are to be returned to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190) for distribution to the Biohazards Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or biosafety@uwo.ca. If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification form. See website: www.uwo.ca/humanresources/biosafety

PRINCIPAL INVESTIGATOR	Joseph Torchia
DEPARTMENT	Oncology, Cancer Research Laboratories, LRCP
ADDRESS	790 Commissioners Rd. E., Room A4-915
PHONE NUMBER	519-685-8692
EMERGENCY PHONE NUMBER(S)	
EMAIL	jtorchia@uwo.ca

Location of experimental work to be carried out: Building(s): LRCP Room(s) A4-915, A4-910, A4-911, A4-917

*For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 15.0, Approvals).

FUNDING AGENCY/AGENCIES: Canadian Institute of Cancer Research (CIHR)
GRANT TITLE(S): _____The role of the ZNF217 oncoprotein in gene regulation and cancer_____

List all personnel working under Principal Investigators supervision in this location:

Name	UWO E-mail Address	Date of Biosafety Training
Majdina Isovic	mbambego@uwo.ca	August 3, 2010
Gobi Thillainadesan	gthillai@uwo.ca	September 2006
Niamh Coughlan	ncoughla@uwo.ca	September 18, 2007
Jennifer Chitilian	jchitili@uwo.ca	Septmber 2009

Please explain the biological agents and/or biohazardous substances used and how they will be stored, used and disposed of. Projects without this description will not be reviewed.

Our laboratory is certified as Level 2 lab under Biosafety Approval Number BIO-LRCC-0005. Adenoviruses used in the lab are commercially available (please see MSDS), and no modifications are being made to the purchased viruses. Viral waste (liquid or solid) is first autoclaved and then disposed of in biohazard waste containers.

For cloning applications we used the DH5α strain *of E.coli,* purchased from Invitrogen. Any liquid waste generated by growing bacterial cultures is bleached and subsequently disposed of. Solid waste is directly disposed of in the biohazardous waste.

Plasmids used for cloning have originally been purchased from different suppliers (please see attached information). Some modifications have been made to these and at present our laboratory has 1200+ different constructs. The original plasmids are modified by insertion of genes of interest. In our laboratory there are two major projects p/CIP and ZNF217 (please see research summary). The newly generated plasmids either have different C or N-terminal tags added to cDNA of interest to allow distinction from the native protein for in overexpression studies. Commonly we also make truncations to cDNA of interest and clone into different plasmid backbones (application dependant transient/stable transfection, protein expression) so that different functional protein regions can be identified. We often resort to mutational studies where binding sites are mutated and downstream changes observed. Any plasmid waste generated is disposed of in biohazardous waste collected by Stericycle, Inc. on daily basis.

Cell lines listed under section 2.3 have all been obtained through a commercial supplier (see attached MSDS). The only modifications made to these cell lines are by transfection of the previously mentioned plasmids so that the cell line would overexpress tagged, mutated or truncated proteins allowing us to study the importance of the proteins in question. Cell line generated liquid waste is bleached and disposed of while solid waste is disposed in biohazardous waste.

The two projects in the lab:

p/CIP

Gene regulation by the ER is a dynamic process involving a carefully orchestrated set of physical and functional interactions between the receptor and a diverse array of transcriptional coregulators. The transcriptional coactivator p/CIP/SRC-3 is among the first coactivators recruited by the ER in response to hormone and plays an integral role in mediating the functional effects of the ER. p/CIP functions primarily as a molecular scaffold that promotes the formation of protein complexes consisting of various enzymatic proteins. The coactivator arginine methyltransferase 1 (CARM1) is one such protein that synergizes with p/CIP at the level of transcription by catalyzing the transfer of a methyl group from S-adenosyl-L-methionine to arginine residues to form methylated arginine derivatives. The present proposal builds on our recent advances on the molecular understanding of p/CIP function in ER signalling events. **The specific hypothesis behind the proposed research is that CARM1 is an essential mediator of p/CIP function which determines both, the transcriptional state of a subset of E2-dependent genes and the oncogenic capacity of p/CIP.** This hypothesis is based on the following observations: First, p/CIP is oncogenic and overexpression of p/CIP has been shown to initiate tumour development in mice. Second, we and others have shown that a functional CARM1/p/CIP complex is rapidly recruited to the pS2 gene, a well-validated ER target gene, in response to hormonal stimulation. Third, CARM1 null mice die at birth and E2-dependent signalling is compromised in the CARM1 null cells. Fourth, we have demonstrated that p/CIP is a substrate for CARM1 both *in vivo* and *in vitro* and methylation affects the stability and transcriptional activity of p/CIP. As part of our longterm goals to understand how defects in hormone-dependent signaling contributes to cancer, the specific objectives of this application are designed to define the gene regulatory function of the p/CIP/CARM1 complex, that may be relevant to aberrant cell proliferation and tumourigenesis

ZNF217

Cancer is a disease involving aberrant cell growth in which the normal control of cell proliferation and division are disrupted. Many proteins that give rise to cancer play an essential role in processes that controls the cells ability to undergo cell division and multiplication. However, when these proteins are mutated, inactivated or amplified, normal growth patterns may be disrupted which could have devastating consequences to the organism. The research in my lab is dedicated to identifying and understanding how specific proteins contribute to the development of cancer. We are focusing our efforts on the analysis of proteins that regulate transcriptional response programs. ZNF217 is a candidate oncogene that is amplified and overexpressed in multiple cancer cell lines and tumours and recent evidence indicates that ZNF217 overproduction results in abnormal cell growth. **The hypothesis behind the proposed research is that deregulated expression of the ZNF217 transcription factor promotes the development of cancer by causing aberrant transcriptional silencing of specific target genes.** This hypothesis is based on the following observations: First, ZNF217 is found within a narrow region of recurrent maximal amplification that is devoid of other transcribed genes. Secondly, we and others have shown that ZNF217 is a major constituent of a transcriptional complex containing chromatin-modifying activities known to promote transcriptional repression. Thirdly, we have identified a consensus recognition sequence suggesting that ZNF217 has DNA binding activity. Fourth, we are combining (1) genome wide expression profiling of cancer cells in which ZNF217 has been downregulated using siRNA and (2) chromatin immunoprecipitation (ChIP) in conjunction with a novel technique, known as DNA selection and ligation (ChIP-DSL), to identify genes directly regulated by ZNF217. Importantly, we have identified and validated the first known targets of the human ZNF217 complex, several of which play a direct role in tumour biology. As part of our long term goals to understand how defects in gene expression pathways contribute to tumourigenesis, the specific objectives of this application are designed to define the role of ZNF217 in transcriptional regulation, as well as explore potential functions that may be relevant to aberrant cell proliferation and cancer.

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? X YES O NO
(non-pathogenic and pathogenic biological agents including but not limited to bacteria and other microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)? If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? O YES X NO
If YES, please give the name of the species. _____
What is the origin of the microorganism(s)? _____

Please describe the risk (if any) of escape and how this will be mitigated:

Please attach the CFIA permit.
Please describe any CFIA permit conditions:

1.2 Please complete the table below:

Name of Biological agent(s)*	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
E.coli (DH5α & BL21)	O Yes X No	O Yes X No	O Yes X No	Frozen stock	Invitrogen	X 1 O 2 O 2+ O 3
Adenovirus	O Yes O No	O Yes O No	O Yes O No		Vector Biolabs	O 1 x 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3

*Please attach a Material Safety Data Sheet or equivalent from the supplier.

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? XYES NO
If no, please proceed to Section 3.0

2.2 Please indicate the type of primary cells (i.e. derived from fresh tissue) that will be grown in culture: **N/A**

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	O Yes O No		Not applicable
Rodent	O Yes O No		
Non-human primate	O Yes O No		
Other (specify)	O Yes O No		

N/A

2.3 Please indicate the type of established cells that will be grown in culture in:

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	x Yes O No	HeLa, MCF7, HaCAT(Line obtained from Lina Dagnino's lab not commercially available) 293T, HT1080	ATCC
Rodent	x Yes O No	mES, MEF	ATCC
Non-human primate	O Yes X No		
Other (Insect cells)	X Yes O No	SF9	ATCC

*Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org) **MSDS not available for HaCAT as obtained from Dagnino Lab on campus.**

2.4 For above named cell types(s) indicate PHAC or CFIA containment level required O 1 X 2 O 2+ O 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? YES x NO
If no, please proceed to Section 4.0

3.2 Indicate in the table below the Human Source Material to be used.

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Infected With An Infectious Agent? YES/NO**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Blood (fraction) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (unpreserved)		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0? x YES NO If no, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? O YES, complete table below O NO

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transfected	Describe the change that results from transformation or tranfection
DH5α	*The lab has about 1200+ different plasmids on hand. Most commonly used backbones are pBKS, pFastback, pCMX,*	*Original constructs have been purchased from various companies, including Promega,*	*ZNf217 and other complex members.* *p/CIP and other complex members*	*The changes done will enable us to generate a tagged protein so that it can be distinguished from the native protein in tissue culture cells or to*

	pcDNA3, pGEX, etc. Backbone used is highly dependant on the application. Transient transfection, stable cell line generation or protein expression.	*Invitrogen, GE Healthcare, and Stratagene.*		*generate a tagged protein in bacteria for subsequent use. Some constructs contain different regions of the protein and these are used to look at changes in interaction with other known interacting proteins. These constructs may influence cell growth and this will be closely monitored.* ***The constructs generated and used in our laboratory are not oncogenic.***

* Please attach a Material Data Sheet or equivalent if available.
** Please attach a plasmid map.

See "New Info"

4.3 Will genetic modification(s) involving viral vectors be made? O YES, complete table below X NO
***We are using commercially available adenovirus and not making changes to the viral vector, for extra information please refer to the attached MSDS.**

Vector(s) *	Virus Used for Vector Construction	Source of Vector	Gene(s) Transduced	Describe the change that results from transduction

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- HIV O YES, please specify _____ X NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ X NO
- SV 40 Large T antigen O YES X NO
- E1A oncogene X YES O NO
- Known oncogenes X YES, please specify _____p/CIP/AIB_____ O NO
- Other human or animal pathogen and or their toxins O YES, please specify _____ X NO

4.5 Will virus be replication defective? X YES O NO

4.6 Will virus be infectious to humans or animals? O YES X NO

4.7 Will this be expected to increase the containment level required? O YES X NO

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? O YES X NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
If no, please proceed to Section 6.0

5.2 If YES, please specify which biological agent will be used: _____
Please attach a full description of the biological agent.

5.2 Will the biological agent be able to replicate in the host? O YES O NO

5.3 How will the biological agent be administered? _____

5.4 Please give the Health Care Facility where the clinical trial will be conducted:_____

5.5 Has human ethics approval been obtained? O YES, number: _____ O NO O PENDING

6.0 Animal Experiments

6.1 Will live animals be used? xYES O NO If no, please proceed to section 7.0

6.2 Name of animal species to be used_____*Mus musculus* (mouse)_____

6.3 AUS protocol # _____2009-052_____

6.4 Will any of the agents listed in section 4.0 be used in live animals O YES, specify: _____ XNO

6.5 Will the agent(s) be shed by the animal: O YES X NO, please justify:

_____

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)? O YES X No If no, please proceed to section 8.0

7.2 Please specify the animal(s) used:

- Pound source dogs O YES O NO
- Pound source cats O YES O NO
- Cattle, sheep or goats O YES, please specify species _____ O NO
- Non-human primates O YES, please specify species _____ O NO
- Wild caught animals O YES, please specify species & colony # _____ O NO
- Birds O YES, please specify species _____ O NO
- Others (wild or domestic) O YES, please specify _____ O NO

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? O YES X NO If no, please proceed to Section 9.0

8.2 If YES, please name the toxin(s)_____
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

8.3 What is the LD_{50} (specify species) of the toxin_____

8.4 How much of the toxin is handled at one time*? _____

8.5 How much of the toxin is stored*? _____

8.6 Will any biological toxins be used in live animals? O YES, Please provide details:_____ O NO

*For information on biosecurity requirements, please see:
http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf

9.0 Insects

9.1 Do you use insects? O YES X NO If no, please proceed to Section 10.0

9.2 If YES, please give the name of the species. _____

9.3 What is the origin of the insect? _____

9.4 What is the life stage of the insect? _____

9.5 What is your intention? O Initiate and maintain colony, give location: _____
O "One-time" use, give location: _____

9.6 Please describe the risk (if any) of escape and how this will be mitigated:
_____
_____

9.7 Do you use insects that require a permit from the CFIA permit? O YES O NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:
_____
_____

10.0 Plants

10.1 Do you use plants? O YES X NO If no, please proceed to Section 11.0

10.2 If YES, please give the name of the species. _____

10.3 What is the origin of the plant? _____

10.4 What is the form of the plant (seed, seedling, plant, tree...)? _____

10.5 What is your intention? O Grow and maintain a crop O "One-time" use

10.6 Do you do any modifications to the plant? O YES O NO
If yes, please describe: _____
_____

10.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:
_____
_____
_____

10.8 Is the CFIA permit attached? O YES O NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:
_____
_____

11.0 Import Requirements

11.1 Will any of the above agents be imported? O YES, please give country of origin_____ X NO
If no, please proceed to Section 12.0

11.2 Has an Import Permit been obtained from HC for human pathogens? O YES O NO

11.3 Has an import permit been obtained from CFIA for animal or plant pathogens? O YES O NO

11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ O NO

12.0 Training Requirements for Personnel Named on Form

All personnel named on the above form who will be using any of the above named agents are required to attend the following training courses given by OHS:

- Biosafety
- Laboratory and Environmental/Waste Management Safety
- WHMIS (Western or equivalent)
- Employee Health and Safety Orientation

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biological agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE _____

13.0 Containment Levels

13.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required. O 1 x 2 O 2+ O 3

13.2 Has the facility been certified by OHS for this level of containment?
X YES, permit # if on-campus BIO-LRCC-0005
O NO, please certify
O NOT REQUIRED for Level 1 containment

Level 2 certified on Dec 10, 2010 by Gail Ryder Gail Ryder

14.0 Procedures to be Followed

14.1 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at http://www.wiph.uwo.ca/

SIGNATURE: _____ Date:2010-08-31

14.2 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, 2+ or 3 measures, that are unique to this agent.

N/A

14.3 Please outline what will be done if there is an exposure to the biological agents listed, such as a needlestick injury:

-Wash the site of injury well under running water

-See Occupational Health and Safety staffed with Registered Nurses for help and to file an incident report

-After hours go to Emergency

15.0 Approvals

1) UWO Biohazards Subcommittee: SIGNATURE: _____
Date: _____

2) Safety Officer for the University of Western Ontario
SIGNATURE: _____
Date: _____

3) Safety Officer for Institution where experiments will take place (if not UWO).
SIGNATURE: Gail Ryder
Date: September 12, 2011

Approval Number: _____ Expiry Date (3 years from Approval): _____

Special Conditions of Approval:

-------- Original Message --------
Subject: Re: Fwd: Fwd: Fwd: Re: Biological Registry Agents Form (Torchia)
Date: Wed, 11 Jan 2012 13:02:38 -0500
From: Majdina Isovic <mbambego@uwo.ca>
To: Jennifer Stanley <jstanle2@uwo.ca>

Hi Jennifer,

Attached are our responses from the last meeting. Please let me know if this is all you require. Please note that we are not generating new Adenovirus, we have only purchased it in the past.

Thanks,
Majdina Isovic

Torchia Lab

1. How is siRNA being delivered?
By oligo

2. Section 4.3 needs to be filled out for the Adenovirus vectors.
There has to be an explicit statement as to what genes are going into the Ad vector.
We are not generating Adenovirus vectors for viral production. We only have purchased virus.

Section 4.6: Yes.

The answer to 4.7 will depend on what type of gene goes in the vector. Is it only Cre? If it is either CIP or ZNF217 (oncogenes),
this may affect the Containment Level requirements.
We are not generating Adenovirus vectors for viral production. We only have purchased virus. Therefore there would be no containment level change. As included in Adenovirus information sheet, biosafety level 2 is required.

3. **Section 4.4:** 293T cells express both T Ag and E1A. Hela are infected with HPV and therefor express their transforming protein.
Other known oncogenes: p/CIP/AIB and ZNF217.

4. Section 11: **Is the company that produces the Ad vector in Canada or out of Canada? If it is not in Canada then a permit may be required.**
The company is located in Philadelphia, PA. All the ordering requirements were followed (ordered 5+ years ago).

Regards
Majdina Isovic

Re: Fwd: Biological Registry Agents Form (Torchia).eml
Subject: Re: Fwd: Biological Registry Agents Form (Torchia)
From: Majdina Isovic <mbambego@uwo.ca>
Date: Wed, 17 Aug 2011 12:30:31 -0400
To: Majdina Isovic <mbambego@uwo.ca>
CC: jtorchia@uwo.ca

New Info

Hi Jennifer,

We finally have all the questions answered, I didn't want to make the changes to the attached pdf as I could see that more recent changes weren't on there.

Thanks for your e-mail. Just a few questions remain from the Committee members. Please make edits and re-send (hopefully for the last time!)

What genes are being expressed to produce recombinant proteins by the baculovirus? Section 4.3 needs to be answered in this regard.

Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results from transduction
Baculovirus	*pFastBac*	*Invitrogen*	*p/CIP, ZNF217*	*Baculovirus generated in our laboratory is used to infect insect cells (SF9) which will generate large amounts of protein of interest. This protein is used in vitro studies. Baculovirus infection of SF9 cells does not make them cancerous or toxic or pathogenic.*

What happens to the cells when infected with Ad? (The question is not who makes it but what is being done with it.)

Cells infected with Adenovirus will express specific protein within the cells to alter their signalling. However, phenotypically, the cells remain the same and 48 hours post infection, the expressed protein will be degraded to restore the original conditions within the cell. Only cells grown in culture conditions can be infected with the adenovirus. Adenovirus does not have the capability to infect regular human cells.

In his description of work (ie with the bactera and viruses), can waste be autoclaved instead of bleached?
Yes, the wasted is autoclaved prior to dispossal.

Section 4.2, needed to add E.coli bore DH5alpha
E.coli DH5alpha

I really hope this is everything I'll have the signature page sent over ASAP.

Thanks,
Majdina

SUMMARY OF THE ANIMAL WORK

Under Animal Use Protocol # 2009-052 (AUS #2009-052) titled "Functional analysis of transcriptional coregulators in the mouse" we currently have a mouse model overexpressing ZNF217 in a tissue specific manner. We are currently expanding the line by breeding the animals containing a single copy of the transgene to *wild-type* animals. Resulting offspring are tagged and tailed at 14 days postpartum, and weaned at 21 days postpartum. Only animals containing the transgene are kept while the *wild-type* mice are sacrificed as explained in the AUS. Line of experimentation involves sacrificing the animal (as described in the AUS) and tissue collection for analysis of transgene expression and its effects on tissues expressed in. All mouse carcasses and tissues are disposed in accordance to the AUS# 2009-052.

BL21

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Bacteria

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Price: $205.00

Organism:	*Escherichia coli* (Migula) Castellani and Chalmers
Designations:	BL21
Depositor:	J Bull
History:	J Bull I J Molineux
Biosafety Level:	1
Shipped:	freeze-dried
Growth Conditions:	ATCC medium129: Nutrient agar with 0.5% NaCl **Temperature:** 37.0°C Duration: aerobic
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
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DH5α

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ATCC® Number:	**68233™** Order this Item	**Price:**	**$200.00**
Designation / Description:	Escherichia coli DH5 alpha, pDSRG, SCC 2197		
U.S. Patent Number:	5,599,906		
Biosafety Level:	1		
Shipped:	room temperature		
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.		

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The Gene Delivery Company

Cre Recombinase

ADENOVIRUS

Product Name: Ad-Cre-GFP

Product Category: human Adenovirus Type5 (dE1/E3)

Description:

Cre Recombinase is a Type I topoisomerase from bacteriophage P1 that catalyzes the site-specific recombination of DNA between loxP sites. loxP is a 34 bp DNA sequence at which confers directionality. Cre recombinase is used as a tool to genetically modify genes, such as to delete a segment of DNA flanked by LoxP sites in cells or experimental animals.

This adenovirus expresses both Cre recombinase and GFP as marker.

Titer: $1x10^{10}$ PFU/ml

Volume: 200ul

Storage Buffer: DMEM with 2% BSA & 2.5% Glycerol

References:

- *Transcription Coactivator Pbp/Med1 Deficient Hepatocytes Are Not Susceptible To Diethylnitrosamine-Induced Hepatocarcinogenesis In The Mouse*
  Carcinogenesis. 2010. 31(2):318-25.
- *Podocytes require the engagement of cell surface heparan sulfate proteoglycans for adhesion to extracellular matrices*
  Kidney International. 2010. XX(XX):XX

Cat. No.	Tag	Promoter	Species	Price **	Availability
1700	GFP	CMV		**$445.00**	In Stock

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3701 Market Street, Ste 434, Philadelphia, PA 19104 | info@vectorbiolabs.com | 877-BIO-LABS (phone) | 215-525-1112 (fax)

VECTOR BIOLABS
THE ADENOVIRUS COMPANY

MATERIAL SAFETY DATA SHEET

EMERGENCY TELEPHONES: 1- 877-Biolabs 1-215-966-6045
http://www.vectorbiolabs.com

MATERIAL SAFETY DATA SHEET - INFECTIOUS SUBSTANCES

SECTION I - INFECTIOUS AGENT

PRODUCT IDENTIFICATION:
All pre-made adenovirus made by Vector BioLabs.

BIOLOGICAL NAME: Adenovirus - Type 5
CHARACTERISTICS: Adenoviridae; non-enveloped, icosahedral virions, 75-80 nm diameter, doubledstranded, linear DNA genome. The recombinant viruses are based on human adenoviral backbone which is deleted in the essential E1 gene as well as the E3 gene. The viruses produced are thus non-replicative.

SECTION II - HEALTH HAZARD

PATHOGENICITY: Varies in clinical manifestation and severity; symptoms include fever, rhinitis, pharyngitis, cough and conjunctivitis. The risk from infection by defective recombinant adenoviral vectors depends both on the dose of virus and on the nature of the transgene. Adenovirus does not integrate into the host cell genome but can produce a strong immune response.
HOST RANGE: Humans and animals
INCUBATION PERIOD: from 1-10 days
MODE OF TRANSMISSION: In the laboratory, care must be taken to avoid spread of infectious material by aerosol, direct contact or accidental injection
CHEMICAL LISTED AS CARCINOGEN OR POTENTIAL CARCINOGEN: None

SECTION III - VIABILITY

DRUG SUSCEPTIBILITY: No specific antiviral available
SUSCEPTIBILITY TO DISINFECTANTS: Susceptible to 1% sodium hypochlorite, 2% glutaraldehyde. Recommend use of 1/3 volume of bleach for 30 minutes.
PHYSICAL INACTIVATION: Sensitive to heat; 1 hour at 56°C is used to inactivate virus.
SURVIVAL OUTSIDE HOST: Adenovirus type 5 survived from 3-8 weeks on environmental surfaces at room temperature.

SECTION IV - MEDICAL

SURVEILLANCE: Monitor for symptoms; confirm by serological analysis
FIRST AID/TREATMENT:
Contact: Immediately flush eyes and skin with plenty of water for at least 15 minutes. Call a physician.
Inhalation: N/A
Ingestion: Wash out mouth with water. Call a physician
Accidental injection: wash area with soap and water. Call a physician.

SECTION V – ACCIDENTAL RELEASE PROCEDURES

Pour 1 volume of Javel water over the leak(s) and wait for 15 minutes.
Wipe up carefully.
Hold for autoclave waste disposal and decontaminate work surfaces with 70% alcohol.

SECTION VI - RECOMMENDED PRECAUTIONS

CONTAINMENT REQUIREMENTS: Biosafety level 2 practices and containment facilities for all activities involving the virus and potentially infectious body fluids or tissues. This level consists of etiological agents considered to be of ordinary potential harm.
PROTECTIVE CLOTHING: Recombinants Adenovirus: Laboratory coat; gloves.

OTHER PRECAUTIONS:
Access to the laboratory is limited.
Work surfaces are decontaminated before and after each procedure
Mechanical pipetting devices are used for all procedures; mouth pipetting is prohibited.
Eating, drinking, and smoking are not permitted in the laboratory; food is not stored in laboratory areas.
Laboratory coats are worn in and are removed before leaving the laboratory.
Hands are washed before and after handling virus.

SECTION VII - HANDLING INFORMATION

DISPOSAL: Decontaminate all wastes before disposal; steam sterilization
STORAGE: In sealed containers that are appropriately labeled

SECTION VIII - MISCELLANEOUS INFORMATION

The above information and recommendations are believed to be accurate and represent the most complete information currently available to us. All materials and components may present unknown hazards and should be used with caution. Vector BioLabs, Inc assumes no liability resulting from use of the above products.

Date of revision: May 24, 2004

3701 Market Street, Suite 340, Philadelphia, PA 19104 Tel: 877-biolabs Fax: 215-966-6001 info@vectorbiolabs.com

ATCC

Sf9

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ATCC® Number:	**PTA-3100™** Order this Item	**Price:**	**$200.00**
Designation / Description:	Insect Cell line, Sf9-P35AcV5-3		
U.S. Patent Number:	7,405,038		
Biosafety Level:	1		
Shipped:	frozen		
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.		

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invitrogen
life

Sf9 Cells (SFM Adapted)
Cat. No. **11496-015**

Adapted to Sf-900 II SFM. (Cat. No. 10902-088)
Sf9 Cells are a clonal isolate, derived from *Spodoptera frugiperda* (Fall Armyworm) IPLB-Sf21-AE cells and are used for transient or stable expression of recombinant proteins. The Sf9 cells are adapted to serum-free suspension culture in Sf-900 II SFM which saves significant time and expense associated with the adaptation of cultures to serum-free and/or suspension cultures (1,2). Insect cells have been historically cultured in stationary systems utilizing T-flasks and serum-supplemented basal medium (3). Insect cells are generally not anchorage dependent and we recommend that the Sf9 seed stocks be maintained in suspension culture, allowing for more flexibility and ease of use.

Prepared from low-passage cells with documented lineage (45 to 50 total passages and 15 to 20 passages serum-free).

Provided as a frozen stock in Sf-900 II SFM containing 7.5% DMSO.

1.5×10^7 total cells provided.

Can be thawed and used directly in suspension culture for rapid expansion of cell stocks, propagation of baculovirus stocks, and production of recombinant proteins, or used as a monolayer for transfection or plaque assay applications.

Protocols for recovery, culture, and cryopreservation are provided on the product insert

Application(s): For baculovirus propagation and recombinant protein expression
Performance testing: Each lot is tested for growth and viability post recovery from cryopreservation. The Master Seed Bank has been tested for viruses, mycoplasma and sterility and identity confirmed by isozyme analysis and karyotyping.
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Sf9 Cells, SFM Adapted

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Cat. No.	11058-021	Cat. No.	62-6520	Cat. No.	10902-096
Unit Size	500 ml	Unit Size	1 ml	Unit Size	500 ml
Price(CAD)	73.00	Price(CAD)	217.00	Price(CAD)	61.95
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MEF (DR4)

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Cell Biology

ATCC® Number: SCRC-1045™ Order this Item **Price:** $249.00

Designations:	MEF (DR4)
Depositors:	ATCC
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Mus musculus* (mouse)
Morphology:	fibroblast
Source:	**Organ:** embryo **Cell Type:** fibroblast
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Restrictions:	Prior to purchase, for-profit commercial institutions must obtain a license agreement. For instructions on how to proceed, please contact ATCC's Office of Licensing and Business Development at licensing@ATCC.org or 703-365-2773.
Isolation:	**Isolation date:** 2003
Applications:	can be used to produce feeder cells
Age:	14 days gestation embryo
Gender:	male and female mixed
Comments:	The cell line was established by ATCCin 2003 from embryonic day 14 (E14) DR4 mouse embryos obtained from The Jackson Laboratory (Stock #003208). Mouse embryo fibroblasts (MEFs) prepared from the DR4 mouse are resistant to common concentrations of the drugs G418, 6-thioguanine, puromycin, and hygromycin. The DR4 strain of mice was developed by Rudolf Jaenisch at the Massachusetts Institute of Technology. The DR4 strain was prepared by the intercrossing of three different strains, one bearing resistance genes neoR and puroR, a second bearing the resistance gene hygR, and a third bearing a natural deletion encompassing the Hprt gene. A series of matings incorporated all 4 drug resistance genes into the strain [PubMed: 9278500]. The original DR4 strain was of mixed background (129/SvJae, 129/OlaHsd, BALB/c, and C57BL/6). The cells can be used as a feeder layer to support the growth of embryonic stem (ES) cells and for the maintenance of ES cells in the undifferentiated state. The growth of these cells should be arrested before being used as a feeder layer. ATCChas successfully irradiated and treated the cells with Mitomycin C for use as a feeder layer. If the MEFs are being used as a feeder layer for ES cells, it is not recommended to use them past passage no. 7 (P7). ATCCtested that this cell line is resistant to: • G 418 (neomycin): 200 microgm/ml • Puromycin: 0.4 microgm/ml • Hygromycin: 110 microgm/ml • 6-Thioguanine: 2.5 microgm/ml
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: • fetal bovine serum to a final concentration of 15% **Atmosphere:** air, 95%; carbon dioxide (CO_2), 5% **Temperature:** 37.0°C
Subculturing:	**Establishing cultures from frozen cells:** To insure the highest level of viability, pre-warm culture medium to 37.0°C before use. Flasks do not need to be coated before plating MEFs. 1. Thaw the vial by gentle agitation in a 37.0°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. 2. Remove the vial from the water bath as soon as the contents are half way thawed (approximately 90 seconds), and decontaminate by dipping in or spraying with 70% ethanol. All

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mES-R1

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Cell Biology

ATCC® Number: SCRC-1011™ Order this Item **Price:** $649.00

Designations:	R1
Depositors:	A Nagy
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Mus musculus* (mouse)
Morphology:	spherical colony
Source:	**Strain:** 129X1 x 129S1 **Organ:** embryo **Tissue:** inner cell mass **Cell Type:** embryonic stem cell;
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Restrictions:	Prior to purchase, for-profit commercial institutions must obtain a license agreement. For instructions on how to proceed, please contact ATCC's Office of Licensing and Business Development at licensing@ATCC.org or 703 365 2773.
Isolation:	**Isolation date:** August, 1991
Age:	3.5 days embryo, blastocyst
Gender:	male
Comments:	The R1 cell line was established in August 1991, from a 3.5 day blastocyst produced by crossing two 129 substrains (129S1/SvImJ and 129X1/SvJ). The cells are heterozygous for the c locus (+/c (ch)) and for the pink eye locus (+/p). In the F1 generation the coat color is uniform agouti, while in the F2 these two coat color genes segregate. The segregation could result in several coat types, from albino, through light brown, to black, depending on the genetic background of the partner of the germline chimaera. Pluripotency of R1 was initially tested by tetraploid embryo <-> ES aggregates for completely ES derived development [PubMed: 8378314]. They were also tested by diploid embryo <-> ES aggregates and blastocyst injection for germline transmission in chimeras [PubMed: 8361547]. At early passages (up to passage #14), one third of the completely R1-derived newborns generated by tetraploid embryo <-> R1 aggregates survived. No live offspring were produced from cells older than passage #14.* . However, about 20% of subclones derived from passage #14 had the original developmental potential of R1 when tested by tetraploid aggregates [PubMed: 8378314]. R1-derived animals reached adulthood and were fertile. The genetically altered lines derived from R1 gave high efficiency of germline transmission either by injecting them into C57 blastocyst or aggregating them with CD-1 or ICR outbred 8-cell stage embryos. More than 90% of the individual K.O. clones went to germline (n>60) by aggregation chimeras. **Current ATCCstocks of R1 cells are beyond passage 14. Current stocks of alternative subclone of R1 cells, designated R1/E (ATCCSCRC-1036), are below passage 14 and have been shown to be germline competent.*
Propagation:	**ATCC complete growth medium:** ES-DMEM (ATCC SCRR-2010) supplemented with 2.0 mM L-Alanyl-L-Glutamine (ATCC 30-2115), 0.1 mM non-essential Amino Acids (ATCC 30-2116), 0.1 mM 2-mercaptoethanol (Invitrogen Life Technologies No. 21985), 1000 U/ml mouse leukemia inhibitory factor (LIF) (Chemicon No. ESG1107) and 15% fetal bovine serum (ATCC SCRR-30-2020). **Atmosphere:** air, 95%; carbon dioxide (CO2), 5% **Temperature:** 37.0°C
Subculturing:	**Protocol:** Establishing and maintaining your culture:To insure the highest level of viability, be sure to warm media to 37°C before using it on the cells. 1. Plate mitotically arrested MEF (CF-1) (ATCCSCRC-1040) as a feeder layer at approximately 1.5 to 2.0 X 10(6) cells/T25 at least one day before plating R1 cells (see product sheet for mitotically arrested MEF for protocol). One hour before thawing the vial of R1 ES cells, perform a 100% medium change using

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HT-1080

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Cell Biology

ATCC® Number: CCL-121™ Order this Item **Price:** $272.00

Designations:	HT-1080
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial
Source:	**Tissue:** connective tissue **Disease:** fibrosarcoma
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Isolation:	**Isolation date:** July, 1972
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
Virus Susceptibility:	Human poliovirus 1 RD-114 Feline Feline leukemia virus Vesicular stomatitis virus
Tumorigenic:	Yes
Oncogene:	ras +
DNA Profile (STR):	Amelogenin: X,Y CSF1PO: 12 D13S317: 12,14 D16S539: 9,12 D5S818: 11,13 D7S820: 9,10 THO1: 6 TPOX: 8 vWA: 14,19
Cytogenetic Analysis:	modal number = 46; range = 44 to 48. Pseudodiploidy was frequently noted. About 40% of the cells had rearranged karyotypes with an extra E-group chromosome and a group C chromosome, probably chromosome 11, was missing.
Isoenzymes:	G6PD, B
Age:	35 years
Gender:	male
Ethnicity:	Caucasian
Comments:	The cells contain an activated N-ras oncogene.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Temperature:** 37.0°C
Subculturing:	**Protocol:**

1. Remove and discard culture medium.
2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes).
   Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal.
4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting.
5. Add appropriate aliquots of the cell suspension to new culture vessels.
6. Incubate cultures at 37°C.

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Cell Biology

ATCC® Number: CRL-11268™ Order this Item **Price:** $272.00

Designations:	293T/17 [HEK 293T/17]
Depositors:	Rockefeller Univ.
Biosafety Level:	2 [Cells contain Adeno and SV-40 viral DNA sequences]
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial
Source:	**Organ:** kidney
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Restrictions:	The line is available with the following restriction: 1. The cell line was deposited at the ATCCby Rockefeller University and is provided for research purposes only. Neither the cell line nor the products derived from it may be sold or used for commercial purposes. Nor can the cells be distributed to third parties for purposes of sale, or producing for sale, cells or their products. The cells are provided as a service to the research community. They are provided without warranty of merchantability or fitness for a particular purpose or any other warranty, expressed or implied. 2. Any proposed commercial use of the cells, or their products, must first be negotiated with Cell Genesys, 500 Forbes Boulevard, South San Francisco, CA 94080 Attn: Robert H. Tidwell; Senior Vice President, Corporate Development.
Antigen Expression:	SV40 T antigen [45408]
Age:	fetus
Comments:	The 293T/17 cell line is a derivative of the 293T (293tsA1609neo) cell line. 293T is a highly transfectable derivative of the 293 cell line into which the temperature sensitive gene for SV40 T-antigen was inserted. 293T cells were cloned and the clones tested with the pBND and pZAP vectors to obtain a line capable of producing high titers of infectious retrovirus, 293T/17. These cells constitutively express the simian virus 40 (SV40) large T antigen, and clone 17 was selected specifically for its high transfectability.293T/17 cells were cotransfected with the pCRIPenv- and the pCRIPgag-2 vectors to obtain the ANJOU 65 (see ATCCCRL-11269) cell line.ANJOU 65 cells were cotransfected with the pCRIPgag-2 and pGPT2E vectors to obtain the BOSC 23 (see ATCCCRL-11270) ecotropic envelope-expression packaging cell line.ANJOU 65 cells were also cotransfected with the pCRIPAMgag vector along with a plasmid expressing the gpt resistance gene to obtain the Bing (see ATCCCRL-11554) amphotropic envelope-expression packaging cell line.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Dulbecco's Modified Eagle's Medium, Catalog No. 30-2002. To make the complete growth medium, add the following components to the base medium: fetal bovine serum to a final concentration of 10%. **Temperature:** 37.0°C **Atmosphere:** air, 95%; carbon dioxide (CO_2), 5%
Subculturing:	**Protocol:** 1. Remove and discard culture medium. 2. Briefly rinse the cell layer with 0.25% (w/v) Trypsin- 0.53 mM EDTA solution to remove all traces of serum that contains trypsin inhibitor. 3. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37°C to facilitate dispersal. 4. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. 5. Add appropriate aliquots of the cell suspension to new culture vessels. 6. Incubate cultures at 37°C.

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ATCC® Number: CCL-2™ Order this Item **Price:** $256.00

Designations:	HeLa
Depositors:	WF Scherer
Biosafety Level:	2 [Cells contain human papilloma virus]
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial
	
Source:	**Organ:** cervix **Disease:** adenocarcinoma **Cell Type:** epithelial
Cellular Products:	keratin Lysophosphatidylcholine (lyso-PC) induces AP-1 activity and c-jun N-terminal kinase activity (JNK1) by a protein kinase C-independent pathway [26623]
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host ([21491] Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents) screening for Escherichia coli strains with invasive potential [21447] [21491]
Virus Susceptibility:	Human adenovirus 3 Encephalomyocarditis virus Human poliovirus 1 Human poliovirus 2 Human poliovirus 3
DNA Profile (STR):	Amelogenin: X CSF1PO: 9,10 D13S317: 12,13.3 D16S539: 9,10 D5S818: 11,12 D7S820: 8,12 THO1: 7 TPOX: 8,12 vWA: 16,18
Cytogenetic Analysis:	Modal number = 82; range = 70 to 164. There is a small telocentric chromosome in 98% of the cells. 100% aneuploidy in 1385 cells examined. Four typical HeLa marker chromosomes have been reported in the literature.HeLa Marker Chromosomes: One copy of MI, one copy of M2, four-five copies of M3, and two copies of M4 as revealed by G-banding patterns. M1 is a rearranged long arm and centromere of chromosome 1 and the long arm of chromosome 3. M2 is a combination of short arm of chromosome 3 and long arm of chromosome 5. M3 is an isochromosome of the short arm of chromosome 5. M4 consists of the long arm of chromosome 11 and an arm of chromosome 19.
Isoenzymes:	G6PD, A
Age:	31 years adult
Gender:	female
Ethnicity:	Black
HeLa Markers:	Y
Comments:	The cells are positive for keratin by immunoperoxidase staining. HeLa cells have been reported to contain human papilloma virus 18 (HPV-18) sequences. P53 expression was reported to be low, and normal levels of pRB (retinoblastoma suppressor) were found.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following

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MCF-7

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Product Description

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Customers in Europe, Australia, Canada, China, Hong Kong, India, Israel, Japan, Korea, Macau, Mexico, New Zealand, Singapore, and Taiwan, R.O.C. must contact a local distributor for pricing information and to place an order for ATCC cultures and products.

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Cell Biology

ATCC® Number: HTB-22™ Order this Item **Price:** $272.00

Designations:	MCF7
Depositors:	CM McGrath
Biosafety Level:	1
Shipped:	frozen
Medium & Serum:	See Propagation
Growth Properties:	adherent
Organism:	*Homo sapiens* (human)
Morphology:	epithelial
	
Source:	**Organ:** mammary gland; breast **Disease:** adenocarcinoma **Derived from metastatic site:** pleural effusion **Cell Type:** epithelial
Cellular Products:	insulin-like growth factor binding proteins (IGFBP) BP-2; BP-4; BP-5
Permits/Forms:	In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.
Applications:	transfection host (Nucleofection technology from Lonza Roche FuGENE® Transfection Reagents)
Receptors:	estrogen receptor, expressed
Antigen Expression:	Blood Type O; Rh+
DNA Profile (STR):	Amelogenin: X CSF1PO: 10 D13S317: 11 D16S539: 11,12 D5S818: 11,12 D7S820: 8,9 THO1: 6 TPOX: 9,12 vWA: 14,15
Cytogenetic Analysis:	modal number = 82; range = 66 to 87. The stemline chromosome numbers ranged from hypertriploidy to hypotetraploidy, with the 2S component occurring at 1%. There were 29 to 34 marker chromosomes per S metaphase; 24 to 28 markers occurred in at least 30% of cells, and generally one large submetacentric (M1) and 3 large subtelocentric (M2, M3, and M4) markers were recognizable in over 80% of metaphases. No DM were detected. Chromosome 20 was nullisomic and X was disomic.
Isoenzymes:	AK-1, 1 ES-D, 1-2 G6PD, B GLO-I, 1-2 PGM1, 1-2 PGM3, 1
Age:	69 years adult
Gender:	female
Ethnicity:	Caucasian
Comments:	The MCF7 line retains several characteristics of differentiated mammary epithelium including ability to process estradiol via cytoplasmic estrogen receptors and the capability of forming domes.The cells express the WNT7B oncogene [PubMed: 8168088]. Growth of MCF7 cells is inhibited by tumor necrosis factor alpha (TNF alpha). Secretion of IGFBP's can be modulated by treatment with anti-estrogens.
Propagation:	**ATCC complete growth medium:** The base medium for this cell line is ATCC-formulated Eagle's Minimum Essential Medium, Catalog No. 30-2003. To make the complete growth medium, add the following components to the base medium: 0.01 mg/ml bovine insulin; fetal bovine serum to a final concentration of 10%

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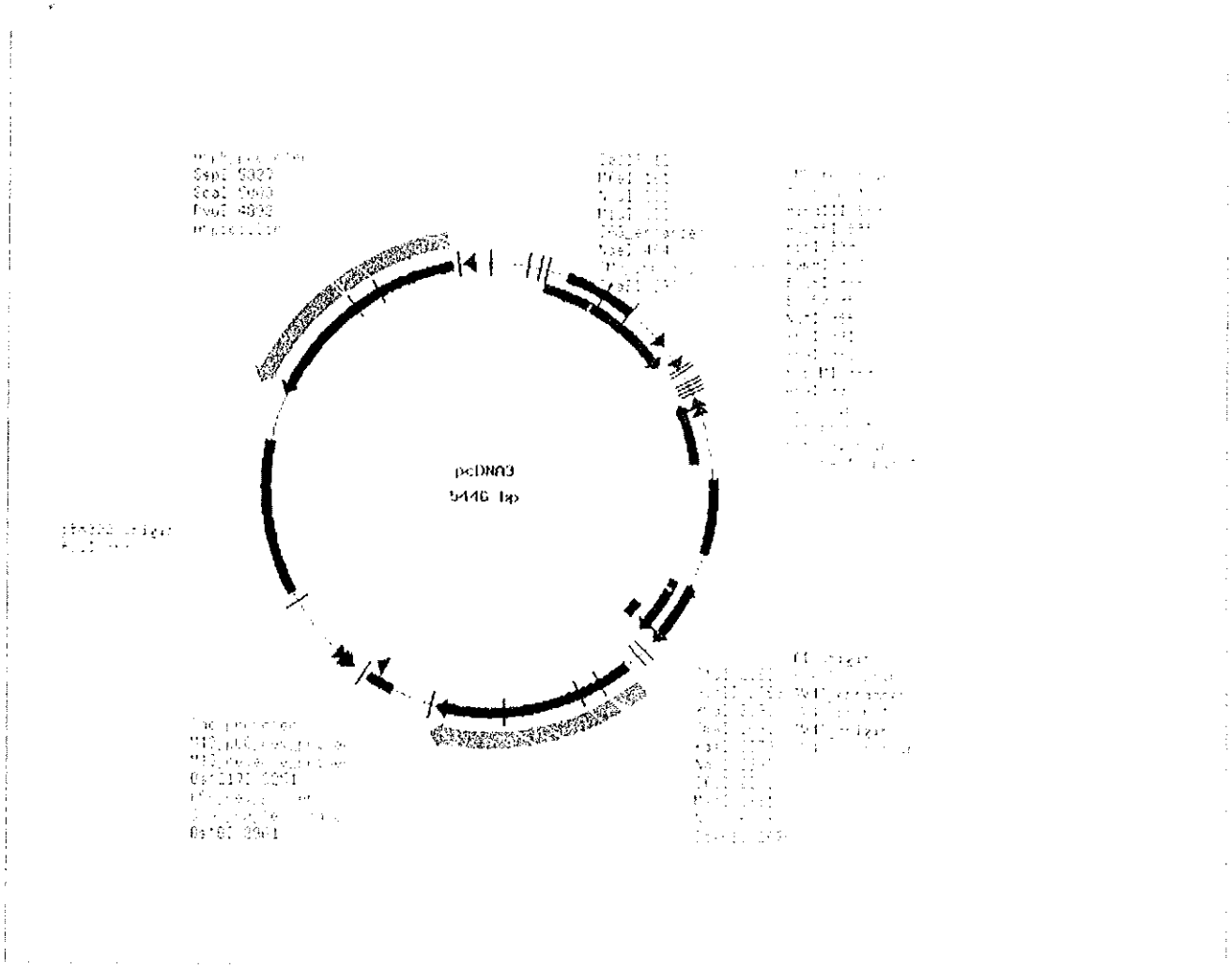
Vector Database > pcDNA3

addgene **Vector Database**

Vector Database is a list of plasmid backbones from publications and several companies, including cloning, mammalian expression, bacterial expression, and lentiviral and retroviral plasmids. The database is compiled by Addgene, and hosted on LabLife. LabLife does not sell or distribute any of the plasmids listed in this catalog.

Plasmid Name	pcDNA3
Source/Vendor	Invitrogen
Plasmid Type	Mammalian expression
Promoter	CMV
Plasmid Size	5446
Sequencing Primer	T7
Bacterial Resistance	Ampicillin
Mammalian Selection	Neomycin
Plasmid Sequence	View Sequence


pcDNA3
5446 bp

invitrogen
life

pFastBac Dual
Cat. No. **10712-024**

The pFastBac™ Dual vector features two promoters in a single vector for expression of two proteins simultaneously in insect cells when using the Bac-to-Bac® Baculovirus Expression System. The vector has two strong promoters, the polyhedrin promoter More

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Bac-to-Bac Baculovirus Expression System

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Vector Name	Map	Polylinker	Sequence	Restriction
pFastBac Dual-Gus/CAT	PDF		TXT	PDF
pFastBac Dual	PDF	PDF	TXT	PDF

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AmpliTaq Gold® PCR Master Mix, 250 Units/ 5mL		Lipofectamine™ 2000 Transfection Reagent		Dulbecco's Modified Eagle Medium (D-MEM) (1X), liquid (high glucose)	
Cat. No.	4318739	Cat. No.	11668-019	Cat. No.	31053-028
Unit Size	1 kit	Unit Size	1.5 ml	Unit Size	500 ml
Price(CAD)	362.00	Price(CAD)	610.00	Price(CAD)	39.25
Qty 1	Add to Cart	Qty 1	Add to Cart	Qty 1	Add to Cart

Vector Database > pBlueScript SK-

addgene **Vector Database**

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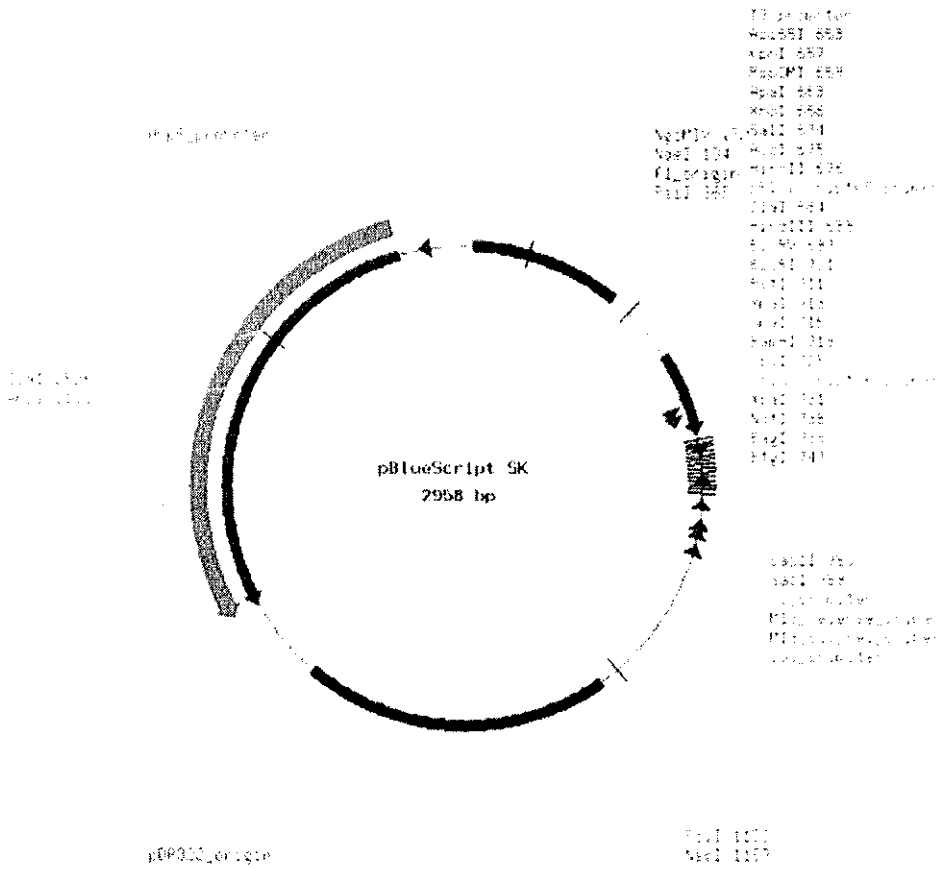
Plasmid Name	pBlueScript SK-
Alt Names	pBSK- , pBSSK
Source/Vendor	Stratagene
Plasmid Type	Bacterial
Plasmid Size	3000
Sequencing Primer	M13/T7/T3
Bacterial Resistance	Amp
Catalog Number	Discontinued
Plasmid Sequence	View Sequence


pBlueScript SK
2958 bp

Vector Database > pCMX

addgene **Vector Database**

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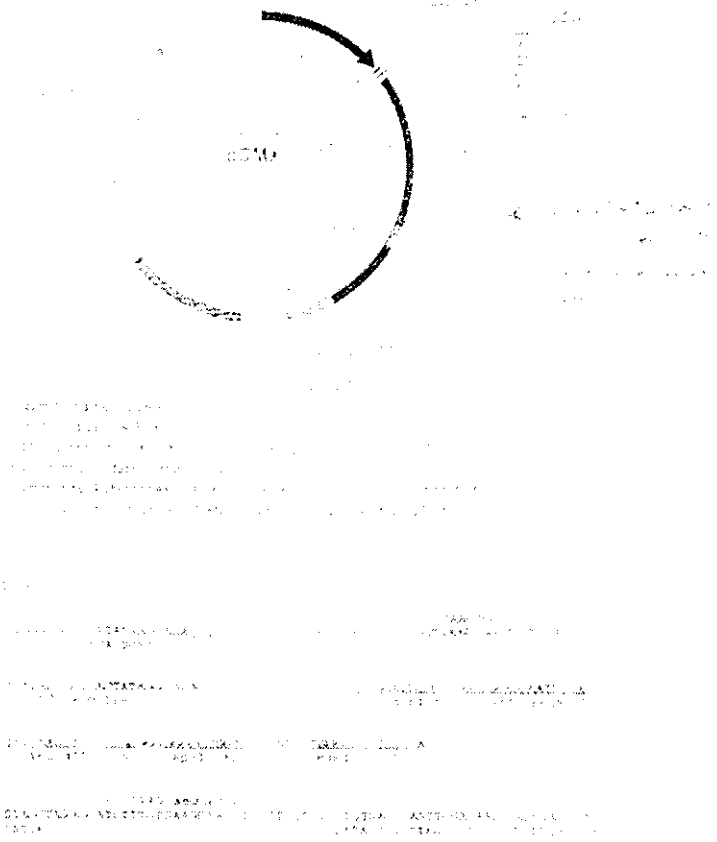
Plasmid Name	pCMX
Plasmid Type	Mammalian
Viral/Non-viral	non-viral
Constitutive/Inducible	constitutive
Promoter	CMV
Plasmid Size	4500
Bacterial Resistance	Ampicillin
Notes	Please see K. Umesono et al., (1991) Cell 65: 1255-1266. The map shown is from Inder Vern lab. The wild-type version of pCMX plasmid does not contain IkB.

Maps



pGEX Vectors (GST Gene Fusion System)

- **A tac promoter for chemically inducible, high-level expression of GST-tagged recombinant proteins.**
- An internal $lacI^q$ gene for use in any E. coli host.
- Very mild elution conditions for release of fusion proteins from the affinity matrix, thus minimizing effects on antigenicity and functional activity.
- PreScission™ Protease, Thrombin, or Factor Xa recognition sites for cleaving the desired protein from the fusion product.

Page down for more information

Order Information

Product	Pack size	Product Code	Price	Qty	
Glutathione S-transferase Gene Fusion Vectors					
pGEX-1λT EcoRI/BAP*	5 µg	28-9546-56	CAN$456.00		add + add to hotlist
pGEX-2T†	25 µg	27-4801-01	CAN$617.00		add + add to hotlist
pGEX-2T*	25 µg	28-9546-53	CAN$671.00		add + add to hotlist
pGEX-2TK†	25 µg	27-4587-01	CAN$678.00		add + add to hotlist
pGEX-3X†	25 µg	27-4803-01	CAN$635.00		add + add to hotlist
pGEX-4T-1†	25 µg	27-4580-01	CAN$617.00		add + add to hotlist
pGEX-4T-1*	25 µg	28-9545-49	CAN$671.00		add + add to hotlist
pGEX-4T-2†	25 µg	27-4581-01	CAN$617.00		add + add to hotlist
pGEX-4T-2*	25 µg	28-9545-50	CAN$671.00		add + add to hotlist
pGEX-4T-3†	25 µg	27-4583-01	CAN$659.00		add + add to hotlist
pGEX-4T-3*	25 µg	28-9545-52	CAN$671.00		add + add to hotlist
pGEX-5X-1†	25 µg	27-4584-01	CAN$659.00		add + add to hotlist
pGEX-5X-1*	25 µg	28-9545-53	CAN$671.00		add + add to hotlist

pGEX-5X-2†	25 µg		27-4585-01	CAN$678.00		add + add to hotlist
pGEX-5X-3†	25 µg		27-4586-01	CAN$678.00		add + add to hotlist
pGEX-6P-1*	25 µg		28-9546-48	CAN$671.00		add + add to hotlist
pGEX-6P-2†	25 µg		27-4598-01	CAN$617.00		add + add to hotlist
pGEX-6P-2*	25 µg		28-9546-50	CAN$671.00		add + add to hotlist
pGEX-6P-3*	25 µg		28-9546-51	CAN$671.00		add + add to hotlist

* E. coli *BL21 is not supplied with the vector. E. coli BL21 can be ordered separately, see E. coli BL21.*
† E. coli *BL21 included with vector.*

You may also need:

Product	Pack size		Product Code	Price	Qty	
2'-Deoxyadenosine 5'-Triphosphate, Disodium, Crystalline (dATP)	250 mg		27-1850-04	CAN$856.00		add + add to hotlist
DRIgest III (λ DNA-*Hind* III/φX-174 RF DNA-*Hae* III Digest)	25 µg		27-4060-01	CAN$255.00		add + add to hotlist
2-D Clean-Up Kit	50 samples		80-6484-51	CAN$370.00		add + add to hotlist
Anti-GST Antibody	0.5 ml, 50 detections		27-4577-01	CAN$445.00		add + add to hotlist
Adenosine 5'-Triphosphate, Disodium, Crystalline (ATP)	25 g		27-1006-03	CAN$526.00		add + add to hotlist
M13KO7 Helper Phage	100 µl		27-1524-01	CAN$86.00		add + add to hotlist

pGEX Vectors (GST Gene Fusion System)

Technical Information

Map of the glutathione S-transferase fusion vectors showing the reading frames and main features. Even though stop codons in all three frames are not depicted in this map, all thirteen vectors have stop codons in all three frames downstream from the multiple cloning site.

Thirteen pGEX vectors are available with or without *E. coli* BL21 (see Figure). Nine of the vectors have an expanded multiple cloning site (MCS) that contains six restriction sites. The expanded MCS facilitates the unidirectional cloning of cDNA inserts obtained from libraries constructed using many available lambda vectors. pGEX-6P-1, pGEX-6P-2, and pGEX-6P-3 each encode the recognition sequence for site-specific cleavage by PreScission™ Protease, (see PreScission Protease) between the GST domain and the multiple cloning site. pGEX-4T-1, pGEX-4T-2, and pGEX-4T-3 are derived from pGEX-2T and contain a Thrombinsee Thrombin recognition site. pGEX-5X-1, pGEX-5X-2, and pGEX-5X-3 are derivatives of pGEX-3X and possess a Factor Xa see Factor Xarecognition site.

Download the pGEX sequence map in PDF format. For ASCII format please scroll down.

pGEX-2TK is uniquely designed to allow the detection of expressed proteins by directly labeling the fusion products *in vitro* (1). This vector contains the recognition sequence for the catalytic subunit of cAMP-dependent protein kinase obtained from heart muscle. The protein kinase site is located between the GST domain and the MCS. Expressed proteins can be directly labeled using protein kinase and [γ-P^{32}]ATP and readily detected using standard radiometric or autoradiographic techniques. pGEX-2TK is a derivative of pGEX-2T; its fusion proteins can be cleaved with Thrombin.

Cleavage of pGEX-6P GST fusion proteins occurs between the Gln and Gly residues of the recognition sequence Leu-Glu-Val-Leu -Phe-Gln-Gly-Pro [(2)]. Low temperature (5°C) digestion minimizes the degradation of the protein of interest. Because PreScission Protease has been engineered with a GST tag, it can also be removed from the cleavage mixture simultaneously with the GST portion of the fusion protein. The pGEX-6P Expression Vectors permit convenient site-specific cleavage and simultaneous purification on Glutathione Sepharose™. The pGEX-6P series provides all three translational reading frames linked between the GST coding region and the multiple cloning site.

Collectively, the pGEX vectors provide all three translational reading frames beginning with the EcoR-I restriction site. pGEX-1λT, pGEX-6P-1, pGEX-4T-1, and pGEX-5X-1 can directly accept and express cDNA inserts isolated from λ gt11 libraries.

Vector	Unformatted	Formatted	GenBank Accession No.
pGEX-1λT, 28-9184-41 AB	*ASCII*	*PDF*	U13849
pGEX-2T, 28-9184-42 AB	*ASCII*	*PDF*	U13850
pGEX-2TK, 28-9189-67 AB	*ASCII*	*PDF*	U13851
pGEX-3X, 28-9184-43 AB	*ASCII*	*PDF*	U13852
pGEX-4T-1, 28-9184-44 AB	*ASCII*	*PDF*	U13853
pGEX-4T-2, 28-9184-45 AB	*ASCII*	*PDF*	U13854
pGEX-4T-3, 28-9184-46 AB	*ASCII*	*PDF*	U13855
pGEX-5X-1, 28-9184-47 AB	*ASCII*	*PDF*	U13856
pGEX-5X-2, 28-9184-48 AB	*ASCII*	*PDF*	U13857
pGEX-5X-3, 28-9184-49 AB	*ASCII*	*PDF*	U13858
pGEX-6P-1, 28-9184-50 AB	*ASCII*	*PDF*	U78872
pGEX-6P-2, 28-9184-51 AB	*ASCII*	*PDF*	U78873
pGEX-6P-3, 28-9184-53 AB	*ASCII*	*PDF*	U78874

Click on "ASCII" to download an unformatted sequence for use by a sequence analysis program. Click on "PDF" to download a formatted sequence and restriction site table. If you prefer accessing the sequence in *GenBank*, refer to the right-hand column for the GenBank accession number:

- **Expression:** Proteins are expressed as fusion proteins with the M_r 26 000 glutathione S-transferase (GST). The GST gene contains an ATG and ribosome-binding site, and is under control of the *tac* promoter. A translation terminator is provided in each reading frame. The resulting fusion protein may be purified using (38861.)
- **Enzymatic cleavage with PreScission Protease:** pGEX-6P-1, -2, -3 allow for removal of the GST carrier protein from the fusion protein by enzymatic cleavage with PreScission Protease. Because PreScission Protease has been engineered with a GST tag, it can also be removed simultaneously with the GST portion of the fusion protein.
- **Enzymatic cleavage with Thrombin:** pGEX-1λT, pGEX-2T, pGEX-2TK, pGEX-4T-1, -2, -3 allow for removal of the GST carrier protein from the fusion protein by enzymatic cleavage with Thrombin.
- **Enzymatic cleavage with Factor Xa:** pGEX-3X, pGEX-5X-1, -2, -3 allow for removal of the GST carrier protein from the fusion protein by enzymatic cleavage with Factor Xa.
- **Direct labeling *in vitro*:** pGEX-2TK allows for direct labeling of fusion proteins *in vitro* with P^{32} using the catalytic subunit of cAMP-dependent protein kinase.
- **Host(s):** *E. coli*. The plasmid provides *lacIq* repressor.
- **Selectable marker(s):** Plasmid confers resistance to 100 µg/ml ampicillin.
- **Amplification:** Recommended.

Properties of pGEX vectors ● Induction: *tac* promoter inducible with 1 to 5 mM IPTG.
● pGEX-1λT Control Regions:

* Glutathione S-transferase gene region: *tac* promoter: -10: 205-211; -35: 183-188; *lac* operator: 217-237; Ribosome binding site

for GST: 244; Start codon (ATG) for GST: 258; Coding region for Thrombin cleavage: 918-935
* MCS: 930-944
* β-lactamase gene region: Promoter: -10: 1308-1313; -35: 1285-1290; Start codon (ATG): 1355; Stop codon (TAA): 2213
* *lacIq* gene region: Start codon (GTG): 3296; Stop codon (TGA): 4376
* Plasmid replication region: Site of replication initiation: 2973; Region necessary for replication: 2280-2976
* Sequencing primers: 5' pGEX Sequencing Primer binds nucleotides 869-891; 3' pGEX Sequencing Primer binds nucleotides 1019-997

• pGEX-2T Control Regions:

* Glutathione S-transferase gene region: *tac* promoter: -10: 205-211; -35: 183-188; *lac* operator: 217-237; Ribosome binding site for GST: 244; Start codon (ATG) for GST: 258; Coding region for Thrombin cleavage: 918-935
* MCS: 930-945
* β-lactamase gene region: Promoter: -10: 1309-1314; -35: 1286-1291; Start codon (ATG): 1356; Stop codon (TAA): 2214
* *lacIq* gene region: Start codon (GTG): 3297; Stop codon (TGA): 4377
* Plasmid replication region: Site of replication initiation: 2974; Region necessary for replication: 2281-2977
* Sequencing primers: 5' pGEX Sequencing Primer binds nucleotides 869-891; 3' pGEX Sequencing Primer binds nucleotides 1020-998

• pGEX-2TK Control Regions:

* Glutathione S-transferase gene region: *tac* promoter: -10: 205-211; -35: 183-188; *lac* operator: 217-237; Ribosome binding site for GST: 244; Start codon (ATG) for GST: 258; Coding region for Thrombin cleavage: 918-935;
* Coding for kinase recognition site: 936-950
* MCS: 951-966
* β-lactamase gene region: Promoter: -10: 1330-1335; -35: 1307-1312; Start codon (ATG): 1377; Stop codon (TAA): 2235
* *lacIq* gene region: Start codon (GTG): 3318; Stop codon (TGA): 4398
* Plasmid replication region: Site of replication initiation: 2995; Region necessary for replication: 2302-2998

Sequencing primers: 5' pGEX Sequencing Primer binds nucleotides 869-891; 3' pGEX Sequencing Primer binds nucleotides 1041-1019

• pGEX-3X Control Regions:

* Glutathione S-transferase gene region: *tac* promoter: -10: 205-211; -35: 183-188; *lac* operator: 217-237; Ribosome binding site for GST: 244; Start codon (ATG) for GST: 258; Coding region for Factor Xa cleavage: 921-932
* MCS: 934-949
* β-lactamase gene region: Promoter: -10: 1313-1318; -35: 1290-1295; Start codon (ATG): 1360; Stop codon (TAA): 2218
* *lacIq* gene region: Start codon (GTG): 3301; Stop codon (TGA): 4381
* Plasmid replication region: Site of replication initiation: 2978; Region necessary for replication: 2285-2981
* Sequencing primers: 5' pGEX Sequencing Primer binds nucleotides 869-891; 3' pGEX Sequencing Primer binds nucleotides 1024-1002

• pGEX-4T-1 Control Regions:

* Glutathione S-transferase gene region: *tac* promoter: -10: 205-211; -35: 183-188; *lac* operator: 217-237; Ribosome binding site for GST: 244; Start codon (ATG) for GST: 258; Coding region for Thrombin cleavage: 918-935
* MCS: 930-966
* β-lactamase gene region: Promoter: -10: 1330-1335; -35: 1307-1312; Start codon (ATG): 1377; Stop codon (TAA): 2235
* *lacIq* gene region: Start codon (GTG): 3318; Stop codon (TGA): 4398
* Plasmid replication region: Site of replication initiation: 2995; Region necessary for replication: 2302-2998
* Sequencing primers: 5' pGEX Sequencing Primer binds nucleotides 869-891; 3' pGEX Sequencing Primer binds nucleotides 1041-1019

• pGEX-4T-2 Control Regions:

* Glutathione S-transferase gene region: *tac* promoter: -10: 205-211; -35: 183-188; *lac* operator: 217-237; Ribosome binding site for GST: 244; Start codon (ATG) for GST: 258; Coding region for Thrombin cleavage: 918-935
* MCS: 930-967
* β-lactamase gene region: Promoter: -10: 1331-1336; -35: 1308-1313; Start codon (ATG): 1378; Stop codon (TAA): 2236
* *lacIq* gene region: Start codon (GTG): 3319; Stop codon (TGA): 4399
* Plasmid replication region: Site of replication initiation: 2996; Region necessary for replication: 2303-2999
* Sequencing primers: 5' pGEX Sequencing Primer binds nucleotides 869-891; 3' pGEX Sequencing Primer binds nucleotides 1042-1020

• pGEX-4T-3 Control Regions:

* Glutathione S-transferase gene region: *tac* promoter: -10: 205-211; -35: 183-188; *lac* operator: 217-237; Ribosome binding site for GST: 244; Start codon (ATG) for GST: 258; Coding region for Thrombin cleavage: 918-935
* MCS: 930-965
* β-lactamase gene region: Promoter: -10: 1329-1334; -35: 1306-1311; Start codon (ATG): 1376; Stop codon (TAA): 2234
* *lacIq* gene region: Start codon (GTG): 3317; Stop codon (TGA): 4397
* Plasmid replication region: Site of replication initiation: 2994; Region necessary for replication: 2301-2997
* Sequencing primers: 5' pGEX Sequencing Primer binds nucleotides 869-891; 3' pGEX Sequencing Primer binds nucleotides 1040-1018

• pGEX-5X-1 Control Regions:

* Glutathione S-transferase gene region: *tac* promoter: -10: 205-211; -35: 183-188; *lac* operator: 217-237; Ribosome binding site for GST: 244; Start codon (ATG) for GST: 258; Coding region for Factor Xa cleavage: 921-932
* MCS: 934-969

* β-lactamase gene region: Promoter: -10: 1333-1338; -35: 1310-1315; Start codon (ATG): 1380; Stop codon (TAA): 2238
* *lacIq* gene region: Start codon (GTG): 3321; Stop codon (TGA): 4401
* Plasmid replication region: Site of replication initiation: 2998; Region necessary for replication: 2305-3001
* Sequencing primers: 5' pGEX Sequencing Primer binds nucleotides 869-891; 3' pGEX Sequencing Primer binds nucleotides 1044-1022

• pGEX-5X-2 Control Regions:

* Glutathione S-transferase gene region: *tac* promoter: -10: 205-211; -35: 183-188; *lac* operator: 217-237; Ribosome binding site for GST: 244; Start codon (ATG) for GST: 258; Coding region for Factor Xa cleavage: 921-932
* MCS: 934-970
* β-lactamase gene region: Promoter: -10: 1334-1339; -35: 1311-1316; Start codon (ATG): 1381; Stop codon (TAA): 2239
* *lacIq* gene region: Start codon (GTG): 3322; Stop codon (TGA): 4402
* Plasmid replication region: Site of replication initiation: 2999; Region necessary for replication: 2306-3002
* Sequencing primers: 5' pGEX Sequencing Primer binds nucleotides 869-891; 3' pGEX Sequencing Primer binds nucleotides 1045-1023

• pGEX-5X-3 Control Regions:

* Glutathione S-transferase gene region: *tac* promoter: -10: 205-211; -35: 183-188; *lac* operator: 217-237; Ribosome binding site for GST: 244; Start codon (ATG) for GST: 258; Coding region for Factor Xa cleavage: 921-932
* MCS: 934-971
* β-lactamase gene region: Promoter: -10: 1335-1340; -35: 1312-1317; Start codon (ATG): 1382; Stop codon (TAA): 2240
* *lacIq* gene region: Start codon (GTG): 3323; Stop codon (TGA): 4403
* Plasmid replication region: Site of replication initiation: 3000; Region necessary for replication: 2307-3003
* Sequencing primers: 5' pGEX Sequencing Primer binds nucleotides 869-891; 3' pGEX Sequencing Primer binds nucleotides 1046-1024

Reference

1. Kaelin, W.G. *et al. Cell* ***70***, 351 (1992).

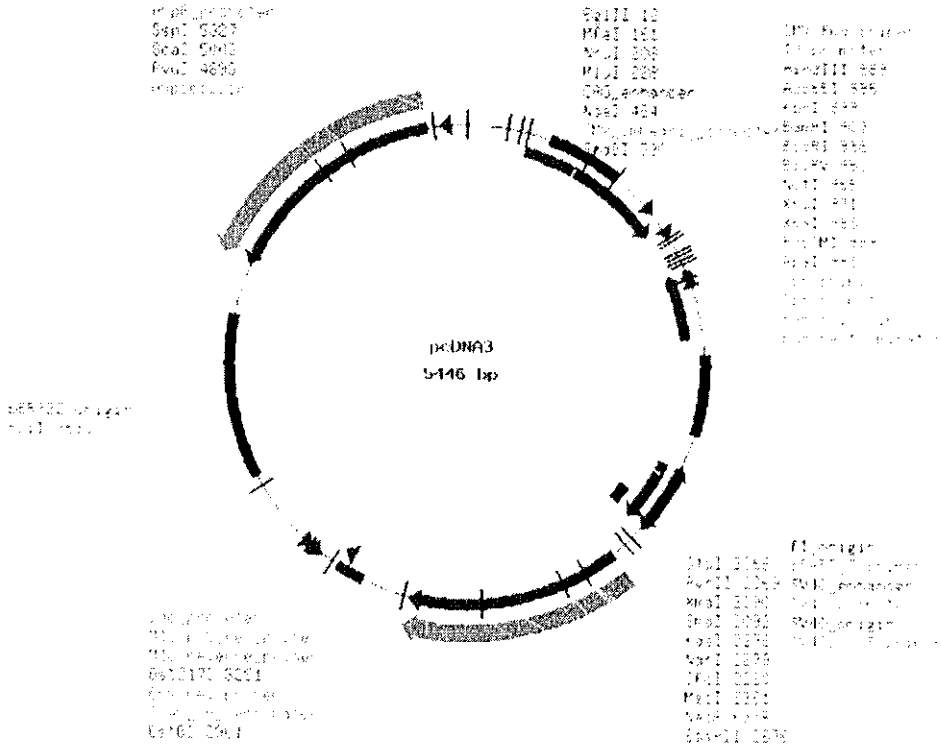
Map of the glutathione S-transferase fusion vectors showing the reading frames and main features. Even though stop codons in all three frames are not depicted in this map, all thirteen vectors have stop codons in all three frames downstream from the multiple cloning site.

[Close]

addgene **Vector Database**

Vector Database is a list of plasmid backbones from publications and several companies, including cloning, mammalian expression, bacterial expression, and lentiviral and retroviral plasmids. The database is compiled by Addgene, and hosted on LabLife. LabLife does not sell or distribute any of the plasmids listed in this catalog.

Plasmid Name	pcDNA3
Source/Vendor	Invitrogen
Plasmid Type	Mammalian expression
Promoter	CMV
Plasmid Size	5446
Sequencing Primer	T7
Bacterial Resistance	Ampicillin
Mammalian Selection	Neomycin
Plasmid Sequence	View Sequence


pcDNA3
5446 bp